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MORPHOLOGY OF THE PUPARIUM OF *LIPOPTENA MAZAMAE* RONDANI (DIPTERA: HIPPOBOSCIDAE)

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Abstract.—The puparium of *Lipoptena mazamae* has an anterior buccal opening with a ventral slit and a posterior region with spiracular pores and an operculated anal area. The spiracular pores have cuticular covering and radiate from each side of the anal area in three curving fields. Each spiracular atrium has a circular opening, minute pegs on the wall, and a tracheal branch beneath. Two triangular, cuticular extensions are situated under the operculum of the anal region. Just below the polygonal area there is an orifice with a collar and an inward cuticular extension. A polygonal pattern that possesses spherical cuticular extensions surrounds the posterior end of the puparium. The remainder of the surface also has a polygonal pattern but with pits on the external and internal surfaces of the cuticle.

Key Words: *Lipoptena mazamae*, Hippoboscidae, puparium, morphology, surface sculpturing, anal opening, respiratory opening

The superfamily Hippoboscoidea (Pupipara) consists of 3 families; Hippoboscidae, Nycteribiidae and Streblidae. The species in these families are obligate ectoparasites and are larviparous. Adult hippoboscids feed on host blood and are known to transmit several parasitic protozoans to domestic and wild birds and mammals (Baker 1967). Bequaert (1953, 1957) gives a detailed description of the anatomy, morphology, biology and evolution of hippoboscids that parasitize mammals and birds. There are several cursory descriptions of the exterior of the puparium of various hippoboscid species (Ferris and Cole 1922, Ferris 1923, 1928, Schruurmans-Stekhoven 1926, Maa 1963, 1969, Theodor 1975). The present study provides a detailed description of the external characteristics of the puparium of *Lipoptena mazamae* Rondani (Diptera: Hippoboscidae).

Materials and Methods

Specimens for light and scanning electron microscopy were taken from white-tail deer, *Odocoileus virginianus* Boddaert, in Mississippi. Ten specimens were used for each technique. It was difficult to work with the puparium because of its deep pigmentation and the deleterious effects of leaving the specimens in KOH until the depigmentation is completed. A simple method for depigmentation of fresh or alcohol stored specimens is given here. First specimens were cut in half and placed overnight in a mixture of 3 ml of distilled water, 3 ml of 30% hydrogen perioxide and 0.2–0.3 ml of concentrated ammonium hydroxide (Stapp and Crumley 1936). Next, they were put in 95% ethanol for 30 minutes and then in two changes of absolute ethanol, also for 30 minutes each and cleared in toluene overnight. Finally the puparia were mounted in a synthetic resin, DPX. Intact puparia were punctured laterally with a minuten. They were put in the depigmenting mixture overnight and then placed in a fresh solution for another 24 h. After dehydration in ethanol the specimens were placed in clove oil for a week. Sometimes the samples had to be placed under a vacuum to facilitate the penetration of the clove oil. Finally the puparia were washed in toluene for 1 h and mounted in Canada balsam.

For scanning electron microscopy fresh or alcohol stored specimens were used. The alcohol stored specimens were rehydrated, then put in 3% OsO_4 for 24 h. They were rinsed for 2 h in four changes of distilled water. After dehydration in ethanol and critical point drying the specimens were attached to aluminium stubs with double-sided sticky tape. The samples were coated with gold-palladium and examined with a Jeol JSM-35CF scanning electron microscope at 20 kV. After the osmication process, several specimens were frozen on dry ice and fractured before further treatment for SEM.

Results

The slightly flattened and oval shaped puparium is 2.3 mm (2.1–2.6 mm) long and 1.9 mm (1.8–2.1 mm) wide (Fig. 1). The anterior end bears a bulge on which the buccal opening is situated and the posterior end bears the spiracular openings and anal operculum (Figs. 1, 3, 5, 11). The buccal opening is circular, with a ventral slit-like extension (Fig. 3).

Just underneath the spiracular and anal region of the posterior end there is a distinct encircling pattern (Fig. 2). The pattern consists of mostly hexagonal polygons with a spherical cuticular extension on each angle (Figs. 2, 4). This pattern is easily observed after the pigmentation is removed. The surface of the rest of the puparium is covered with a similar polygonal pattern (Figs. 4, 10). In addition, there are pits at the angles of each polygon (Fig. 10), and distributed over the inner surface of the polygons (Fig. 8).

Underneath the posterior plate there is a large opening with a distinct collar and surrounding cuticular ridges (Figs. 2, 4, 11). The collar is actually the exposed end of a long internal cuticular extension. Internally there is another collar (Fig. 11 and inset).

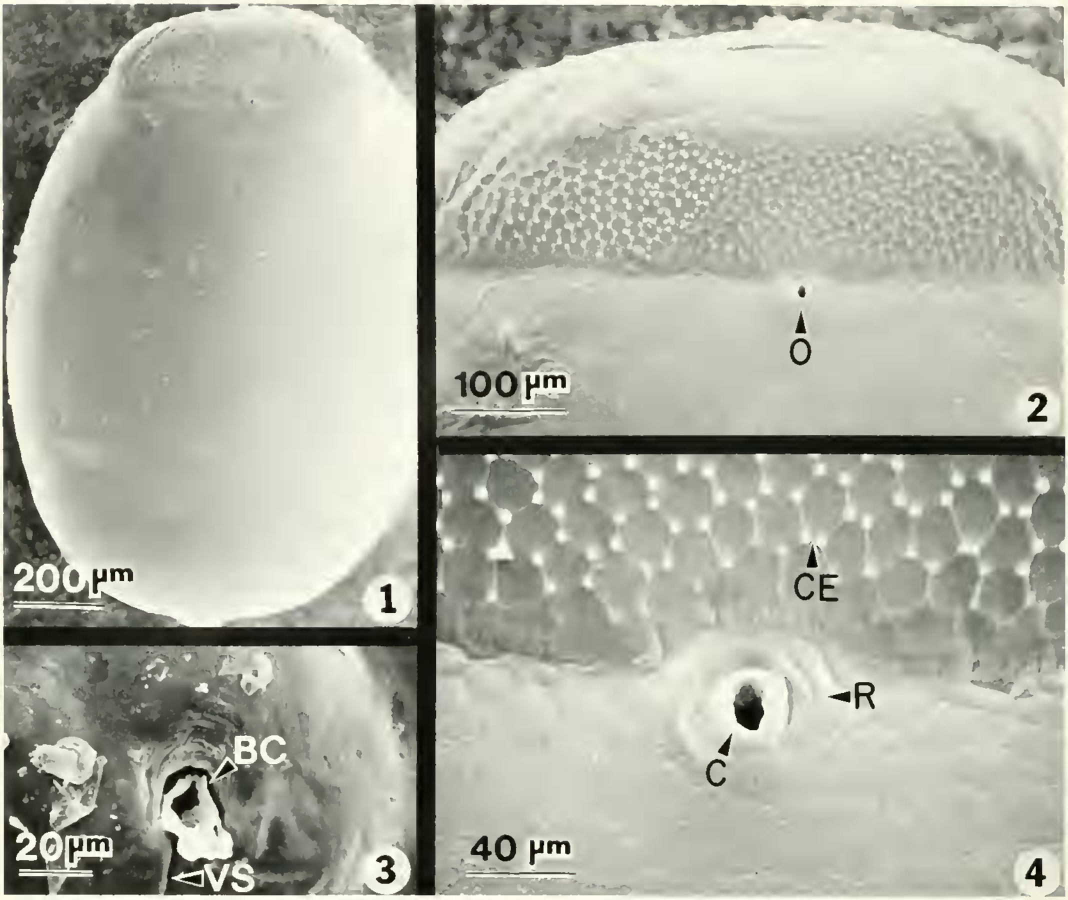
The anal opening, which is situated on the apex of the posterior portion of the body, is hexagonal in shape and has an operculum covers the anal opening (Figs. 5, 11). Beneath the operculum there are two triangular extensions (Fig. 14). Externally the spiracular pores radiate lateral of the anal opening and form three branches on each side with 9, 15 and 17 openings respectively (Figs. 5, 12, 13). Each spiracular pore has an oval-shaped plate with a circular opening (Fig. 6) and under the plate is the slit-like aperture of the atrium (Figs. 7, 8, inset). Inside the atrium there are blunt, oblong projections that line the atrial wall (Fig. 8, inset).

Internally, there is an enlarged portion of the tracheal trunk from which five tracheal branches radiate (Figs. 12, 13). One branch extends back into the body whereas the other four have shorter secondary branches that extend to the spiracular atria (Figs. 7, 8, 12, 13). The internal surfaces of the tracheae have a meshwork of cuticular thickenings (Figs. 7, 9) which maintains shape and provides flexibility to the wall of the tracheae.

Discussion

The size, shape and buccal opening of the puparium is similar in other *Lipoptena* and hippoboscid species (Ferris 1923, 1928, Coatney 1931, Maa 1963, Theodor 1975). According to Bequaert (1953), the developing larva receives its nourishment from the milk glands via the buccal opening.

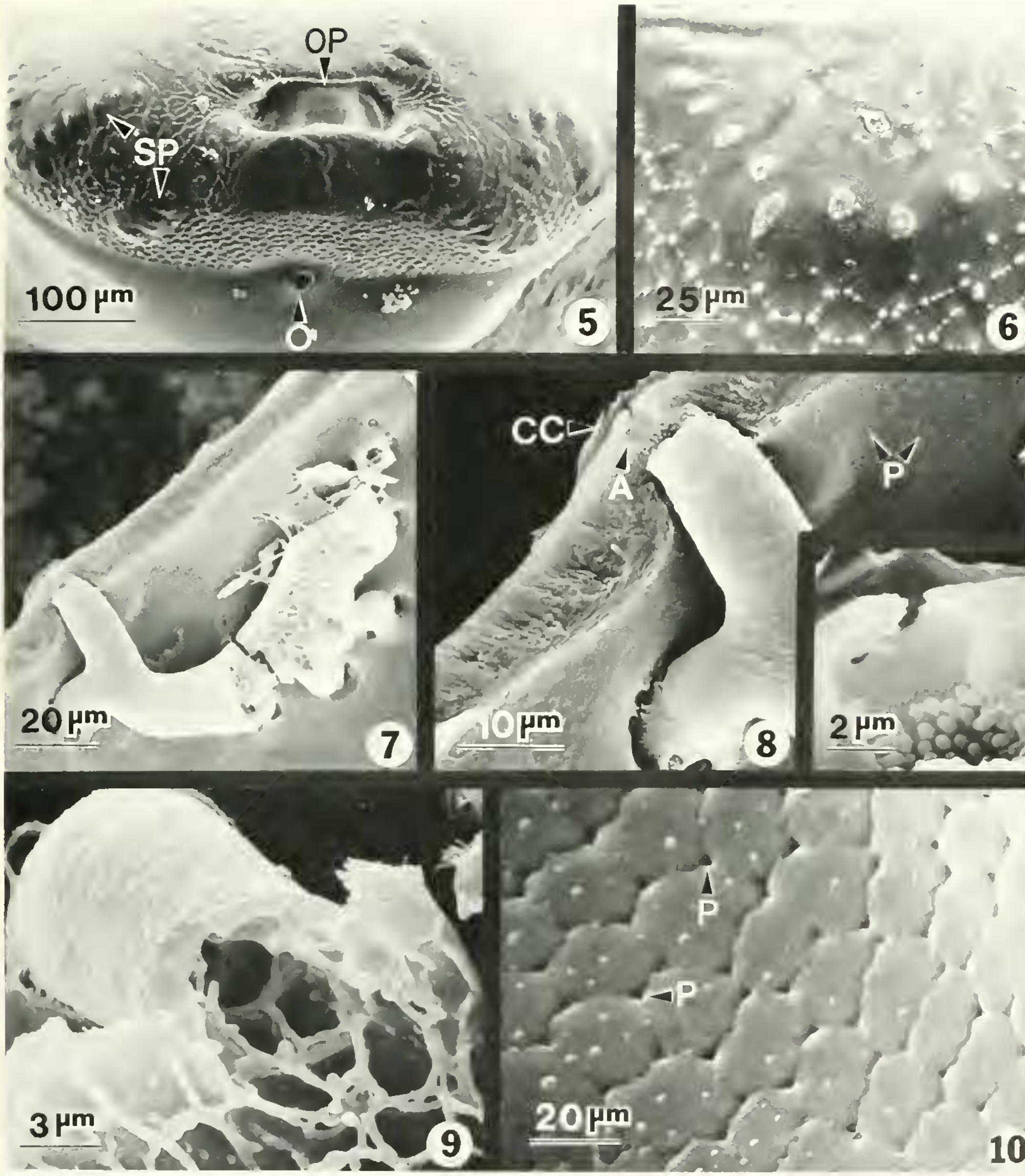
Surface patterns occurs in several genera but the hexagonal pattern with the spherical cuticular extensions that encircles the posterior portion of the puparium in *L. mazamae* is not mentioned in the description



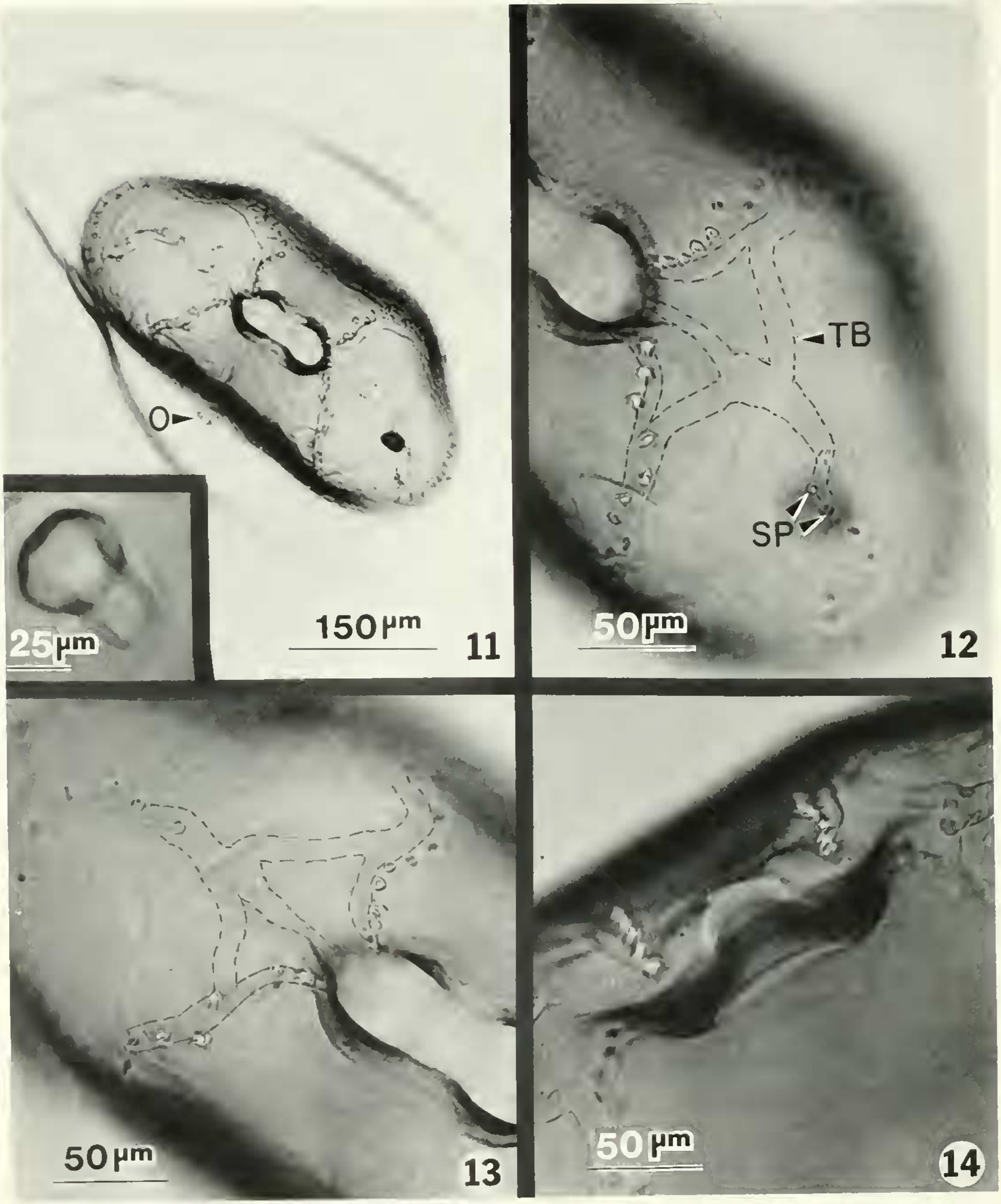
Figs. 1–4. SEM micrographs of the puparium, of *L. mazamae.* 1, Lateral aspect (posterior end shown at top). 2, Posterior region showing the polygonal and orifice (O). 3, Anterior end showing circular buccal cavity (BC) and ventral slit-like extension (US). 4, Higher magnification of Fig. 3 showing circular extension (CE) at the angles of the polygons and the collar (C) and ridges (R) around the orifice.

of a closely related species, *L. depressa,* by Ferris and Cole (1922) and Hearle (1938) or of other genera such as *Pseudolynchia* (Coatney 1931), *Olfersia* (Ferris 1928) and *Hippobosca* (Schruumans-Stekhoven 1926). The rest of the puparium surface in *L. mazamae* has a polygonal pattern with distinct pits. Surface patterning occurs in several genera. Bequaert (1953) observed that the surface in *Stenepteryx* and *Pseudolynchia* are covered with a meshwork of lines whereas in *Stilbometopa* and *Ornitheza* have a punctate surface. The surface in *Olfersia* has straight or hooked spines and short triangular spinules are situated on the surface in *Hippobosca* (Ferris 1928). More research is needed to determine if the differences in surface patterning and sculpturing could be taxonomically important.

Maa (1963, 1969) and Theodor (1975) refer to a large opening below the posterior portion of the puparium as the ventroapical pit, but neither author gives any description of the external area surrounding the opening, or mention the internal cuticular extension. The function of this structure is not known. The shape of the operculum on the anal opening of *L. mazamae* is similar



Figs. 5–10. SEM micrographs of the puparium. 5, Hexagonal operculum (OP) and the arrangement of the respiratory openings (SP). 6, An array of posterior respiratory openings. 7, Tracheal branch under several respiratory openings. 8, Tracheal branch coming from the atrium (A) of the respiratory opening (CC, cuticular covery) (P, pits). Inset, higher magnification of the atrial region, note the peg-like structures on the atrial wall. 9, Inner meshwork of a tracheal branch. 10, Polygonal pattern on the surface of the puparium and the distinct pits (P) associated with the polygons.



Figs. 11–14. Light micrographs of the posterior region of the puparium. 11, Operculum (O) and radiating branches of the tracheal system and the orifice just beneath this region. Inset, the internal cuticular extension of the orifice. 12, 13, Radiating tracheal branches (TB) and respiratory openings laterad of the operculum, (SP, spiracular pore). Dash lines indicate the tracheal branches. 14, Cuticular extension beneath the operculum.

in *Ornithomyia strigilecula* Ferris (Ferris 1923) and in *Hippobosca maculata* Leach (Schruumans-Stekhoven 1926). But previous descriptions of the puparia of *Lipoptena* species do not mention the two triangular extensions beneath the operculum. These structures may be apodemes which attach the muscles that open and close the operculum.

At present there is no description of the external and internal structure of the spiracular pores on the posterior end of a hippoboscid puparium. The outer plate with its narrow opening and the projections on the atrial wall are associated with the tracheal system of other insects and probably act as a filtering system to prevent blockage of the tracheae (Chapman 1982). The internal surface of the tracheae have taenidia which maintain shape and flexibility in the wall of the tracheae (Chapman 1982). The pits on the internal surface of the puparium may be pores that are involved in gas exchange during the pupal stage.

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